

Studies on Macroscopic, Microscopic, and TLC Based Phytochemical Analysis of *Euphorbia thymifolia* Linn.

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ABSTRACT

Euphorbia thymifolia L. (Euphorbiaceae) is a small branched plant. The leaves, seeds and fresh juice of the whole plant are used in worm infections as a stimulant. Phenols are present in large amount in this plant which has played a major role in household's products and as an intermediate for industrial synthesis. The study of macroscopic and microscopic examination of *E. thymifolia* has been proved that this plant has smooth and thin surface. In the TLC method, there were four and five spots were observed which indicated that flavonoid was present in good quality. This fact was also supported by quantitative analysis of flavonoid for *E. thymifolia*. So this study indicated that *E. thymifolia* plant having moderate amount of flavonoids, which can be good source of antioxidants and food supplements.

Key-words: *Euphorbia thymifolia*, Laghududhika, Macroscopic, Microscopic, Phytochemical

INTRODUCTION

Euphorbia thymifolia Linn. usually referred to as laghududhika or choti-dudhi. *E. thymifolia* belongs to the family Euphorbiaceae, which has around 7500 species in about 300 genera. The plants under Euphorbia genus are used to treat cancer, migraine, warts, intestinal parasites, tumours, etc. The *E. thymifolia* is found in tropical regions ^[1] but in India, the plant is found in the hills and plains. The use of *Euphorbia thymifolia* is aromatic, sedative, antiviral, anti-inflammatory, anti-spasmodic, anti-fungal, anti bacterial, and diuretic properties ^[2].

Properties and Uses of *Euphorbia thymifolia*

Medicinal Uses- It is useful in vitiated condition of constipation, helminthiasis and ringworm, skin diseases and leprosy ^[3].

The leaves and seeds were given in worm cases and in certain bowel affections of children. They were considered stimulant and laxative. The 4,5 anti viral activity was proven in experiment and anti microbial activity reported by Gupta *et al.* ^[4].

Antimicrobial Activity- *E. thymifolia* is considered to possess Antimicrobial activity due to the presence of alkaloids. The extracts of *E. thymifolia* were used in drugs like fluconazole and ciprofloxacin to control the microbes ^[5].

Antibacterial, Antifungal and Antiviral Activity- Antibacterial activity was demonstrated using an ethanolic extract of *Euphorbia thymifolia* against *Bacillus pumilis*, *S. aureus*, and *B. Subtilis* ^[6]. *E. thymifolia* is found to have Antifungal activity. Ethanolic extract of *Euphorbia thymifolia* was used against fungal strain *Candida albicans* to study the Antifungal activity. The latex of *Euphorbia thymifolia* is also found to show Antifungal activity. The fungi namely *Aspergillus niger*, *Trichoderma viride*, *Alternaria alternate*, *Fusarium moniliform* and *Curvularia lunata* were found to shown reduced activity when treated with the latex extract of *E. thymifolia* thus proving it to be an effective Antifungal agent. Virus infectivity was significantly reduced in a

How to cite this article

Vaid PK, Kumar A, Singh M, Tyagi V, Kushwaha A. Studies on Macroscopic, Microscopic, and TLC Based Phytochemical Analysis of *Euphorbia thymifolia* Linn. Int. J. Life Sci. Scienti. Res., 2018; 4(3): 1744-1751



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concentration of 4.0 μ /ml of ethyl acetate extract, whereas, 3OG46HG diminished virus infectivity at a concentration of a 0.5 μ /ml^[7].

Anti-spasmodic Activity- Spasm occurs mainly due to overuse of the muscle so that the muscle loses all the energy. The extract of *E. thymifolia*, which was obtained using the ethanol was used to study anti-spasmodic activity and it was observed that the extract could inhibit the growth of *Plasmodium falciparum*.

Anti-hyperglycemic Activity- The oral test method was used to determine the anti-hyperglycemic activity for glucose tolerance. Mice were taken as the study subjects and were injected with different doses of the extract followed by glucose^[8].

Anti-arthritic Activity- Albino rats were used to screen the anti-arthritic activity of *E. thymifolia*, where the aqueous extracts were used. The white blood cells, haemoglobin content, red blood cells, erythrocyte sedimentation rate, total protein, alkaline phosphate, serum glutamic pyruvate transaminase, serum glutamic oxaloacetate transaminase, lipid peroxidation were estimated. This result proved the anti-arthritic activity of *E. thymifolia*^[9].

Anti-inflammatory Activity- Anti-inflammatory activity was studied using ethanolic plant extract of carrageenan-induced rat paw edema method. The reduction in the edema was observed with the dose of 100 mg/kg body weight, when compared to Indomethacin, which is a standard drug (10 mg/kg) and thus the extract produced sufficient anti-inflammatory response^[10].

Diuretic Activity- Diuretic activity of ethanolic extract and fractions of *E. thymifolia* was examined for its diuretic activity by its ethanolic extract and fractions. The dose dependent method was used to determine the diuretic activity^[11].

MATERIALS AND METHODS

This study was performed from Jan to July 2017 at Faculty of Biosciences, Shri Ram College, Muzaffarnagar UP, India.

Apparatus- Test tube, Graduated cylinder, Funnel, Beaker, Pestle and Mortar, Water Bath, Methanol, Measuring cylinder, Soxhlet apparatus, Beaker,

Whatman paper, Distilled water, funnel, eppendorf tube, Flask, Measuring cylinder, Rotary shaker apparatus, Crucible and Water bath.

Plant Materials- The present study was carried out on *E. thymifolia*. This plant is present in the wastelands, along roadsides and wall sides in humid conditions. In India, the plant is found in the hilly and plain areas. *E. thymifolia* is a prostrate annual plant producing stem up to 25 cm long. The stem usually produced numerous adventitious roots.

Collection of Plant- After selection of plant, it was must to collect the plant parts for the research purpose. Throughout India, the plant *Euphorbia thymifolia* is available. After the collection of sample, it needs to be dried to make the sample extract. In general, the plant material should be dried at temperature below 30°C to avoid the decomposition of thermo-labile compounds. Shade dried the sample and powder was prepared with the help of the blender.

Standardization

Macroscopic Examination

(a) Size- A graduated ruler in millimeters was used for measurement of the length, width of crude materials.

(b) Color- Untreated sample was examination under diffused daylight.

(c) Surface characteristics- The material was touched to determine if it is soft or hard; bent and ruptured to obtain information on brittleness and the plant material were fractured to observe whether the material was fibrous, smooth, rough and granular.

(d) Odor- The material was powdered and the strength of the odor was determined whether (weak, distinct, strong) and sensation of odor whether (aromatic, fruity, musty, moldy, rancid etc) was observed.

(e) Taste- The small amount of both plant materials was tested and observation was taken.

Microscopic Examination- Microscopy of Fresh Leaf Stomata, trichomes and epidermal cell are important identifying characteristics of the leaf. In transverse section, their exact nature can't be studied. Hence, exposure of surface/ epidermis becomes important for the detailed microscopic study.

Procedure- The piece of leaf was cleared off by boiling with chloral hydrates and then an upper layer of the epidermis was peeled out. The section of the epidermis was kept on a slide and mounted in glycerine water. Various features of leaf were examined. In case of stomata, stomatal number and stomatal index were taken out by arranging the camera Lucida and drawing board for making the drawing to scale. One mm of square was drawn by means of stage micrometer and then cleared leaf was placed on the slide. The epidermal cells and stomata were traced and then the number of stomata present in the area of 1 sq. mm was counted and stomatal index was calculated by the formula:

$$I = S/E+S \times 100$$

Where, I= stomatal index,

S= No. of stomata per unit area,

E= No. of epidermal cells in the same unit area,

Microscopic examination of the stem- Microscopy of the fresh stem was studied. For microscopy transverse section of the stem was taken and stained with safranin 10. Photomicrographs were obtained from the section. The histochemical analysis was done by staining the hand-cut section with the different reagent. The stems were treated with chloral hydrates solution followed by staining in 1% safranin for 5 to 10 min. and mounted in 15% glycerin ^[12].

Preliminary Screening of Secondary Metabolites

Extraction- The shade dried leaves material was powdered using mixer grinder and subjected to Soxhlet extraction with methanol for 18 hrs. The solvent was evaporated by using steam water bath and extract was weighted. The condensed extract was used for preliminary screening of phytochemicals.

Detection of Alkaloids

Hagar's Test- Filtrates were treated with Hagar's reagent (saturated solution of picric acid solution). Formation of yellow precipitate indicated the presence of alkaloids.

Detection of Carbohydrates

Fehling's Test- Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solution. Formation of a red precipitate indicates the presence of reducing sugars.

Detection of Glycosides

Modified Bontrager's Test- Extracts were treated with ferric chlorides solution and immersed in boiling water for about 5 min. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicated the presence of glycosides.

Detection of Saponins

Foam Test- Small amount of extract was shaken with little quantity of water. If foam produced persists for 10 min, indicated the presence of saponins.

Detection of Phytosterols

Salkowski's Test- Extract was treated with chloroform and filtered. The filtrates were treated with a few drops of conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow color indicated the presence of triterpenes.

Detection of Resins- Extract was treated with acetone. Then small amount of water was added and shaken. The appearance of turbidity was indicated the presence of resins.

Detection of Phenol- Extract was treated with few drops of ferric chloride solution formation of bluish-black color indicated the presence of phenols.

Detection of Tannin- To the extract, gelatin solution (1%) containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

Detection of Flavonoids

Lead Acetate Test- Extract was treated with few drops of leads acetate solution. Formation of yellow color precipitate indicated flavonoids.

Detection of Proteins

Xanthoproteic Test- Extract was treated with few drops of conc. Nitric acid solution. Formation of yellow color indicated the presence of protein.

Detection of Diterpenes

Copper acetate test- Extract was dissolved in water and treated with few drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes ^[13].

TLC- The sample was spotted on the plate and dried for a few minutes. Then the solvent system was prepared and allowed to stabilize for 10 min. Then the plate was dipped in the solvent chamber and allowed to run up to three fourth of the plate. Then it was removed and was air-dried. The plate was examined visually.

RESULTS AND DISCUSSION

This result indicated in this study that *Euphorbia thymifolia* has extreme scope of medicinal as well as anti-aging components. Phytochemical characteristics verified with various test results given below. The quantity of extract was determined with two methods, one with methanol in Soxhlet was found 2.1%, while in

another with methanol in rotary shaking was 2.3%. In the preliminary phytochemical analysis, the phenolic compounds represent good quality while alkaloids, flavonoids, tannins, carbohydrates, glycosides and diterpenes were present in moderate quality and phytosterols and resins show very less quality but proteins and saponins were not found in *E. thymifolia*. Phenols are present in large amount in this plant which is widely used in household products and as intermediate for industrial synthesis. Powder of plant has the various characteristic in which odor was pleasant and bitter in taste.

Table 1: Nature and percentage yield of extracts of *Euphorbia thymifolia*

S. No.	Name of the extract	Nature	Colour	% Yield (w/w)
1.	Methanolic Extract with Soxhlet	Shade	Green	2.1
2.	Rotary shaking extraction	Shade	Green	2.3

Standardization

Macroscopic Examination

Table 2: Macroscopic examination of Leaves powder

S. No.	Organoleptic Characterization	<i>Euphorbia thymifolia</i>
1.	Size	1 cm
2.	Surface Characteristics, texture	Smooth
3.	Taste	Bitter
4.	Colour	Green
5.	Odour	Pleasant

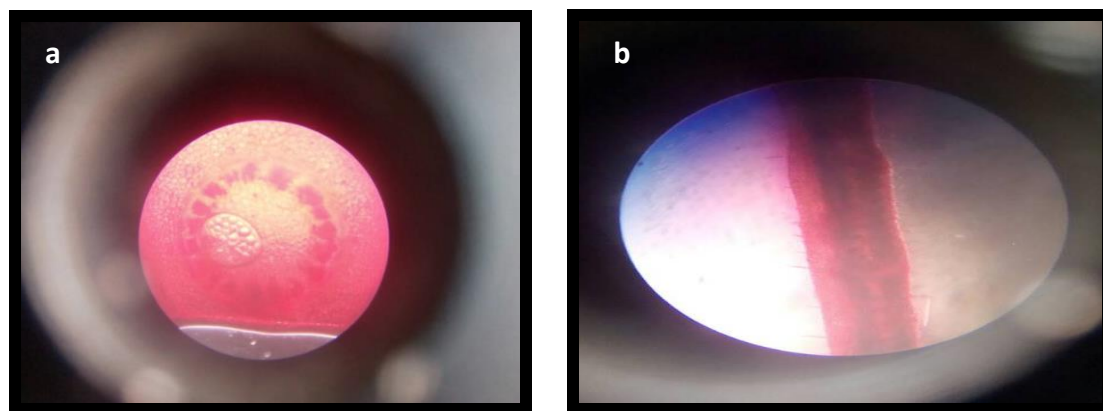


Fig. 1: (a) Transverse Sectioning (TS) of the stem, (b) Transverse sectioning (TS) of leaves of *Euphorbia thymifolia*

Table 3: Phytochemical constitute of *Euphorbia thymifolia*

S. No.	Phytochemical Name	Reach or reagents test	Observation	Test Result
1.	Alkaloids	Hagar’s reagent	Yellow Colour Precipitates	++
2.	Carbohydrates	Fehling’s test	Red colour Precipitates	++
3.	Glycosides	Modified Borntrager’s test	Rose pink colour of ammonical layer	++
4.	Saponins	Foam test	Foam produced	-
5.	Phytosterols	Salkowski’s Test	Appearance of the golden yellow color	+
6.	Resins	Acetone-water test	Appearance of turbidity	+
7.	Phenol	Ferric chloride test	Appearance of the bluish and black color	+++
8.	Tannins	Gelatin test	White colour Precipitates	++
9.	Flavonoids	Lead acetate test	Yellow Colour precipitates	++
10.	Proteins	Xanthoproteic test	Apperance of the Yellow Color	-
11.	Diterpenes	Copper acetate test	Appearance of emerald green color	++

(Absent= -), (Present=+), (Medium concentration=++), (High concentration=+++)

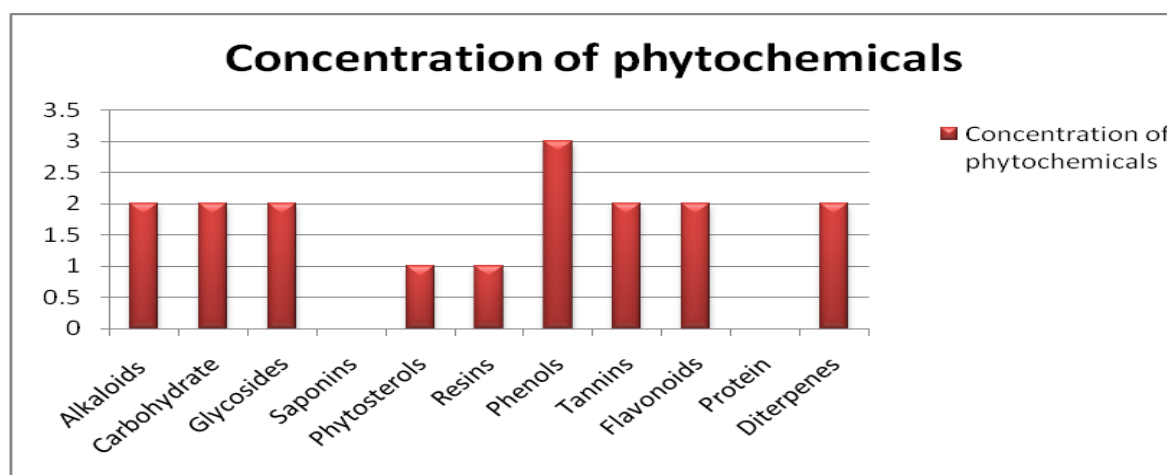


Fig. 2: Phytochemicals conc. of *Euphorbia thymifolia*

Thin layer chromatography (TLC)

Table 4: TLC with solvent system I Chloroform: Methanol: nButanol: Water (10:10:1:6)

Plant species	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	Color	R _f value
<i>E. thymifolia</i>	Methanolic extract	1.6	6	Orange	0.26
		2.5	6	Light Blue	0.41
		2.8	6	Dark Green	0.46
		3.7	6	Light Green	0.61

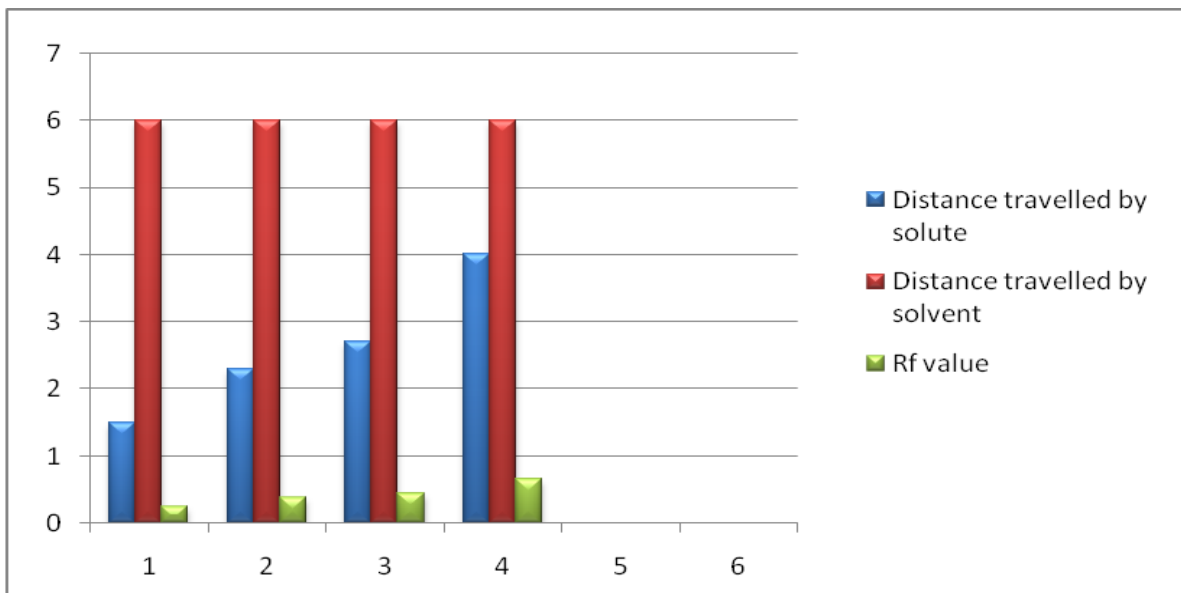


Fig. 3: TLC of flavanoids with solvent system I (Chloroform: Methanol: nButanol: Water (10:10:1:6))

Table 5: TLC with solvent system II nButanol: Ethanol: Water (4:1.5:5)

Plant species	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	Colour	R _f value
<i>Euphorbia thymifolia</i>	Methanol	1.5	6	Orange	0.25
		2.3	6	Light Orange	0.38
		2.7	6	Light Orange	0.45
		4.0	6	Red Orange	0.66

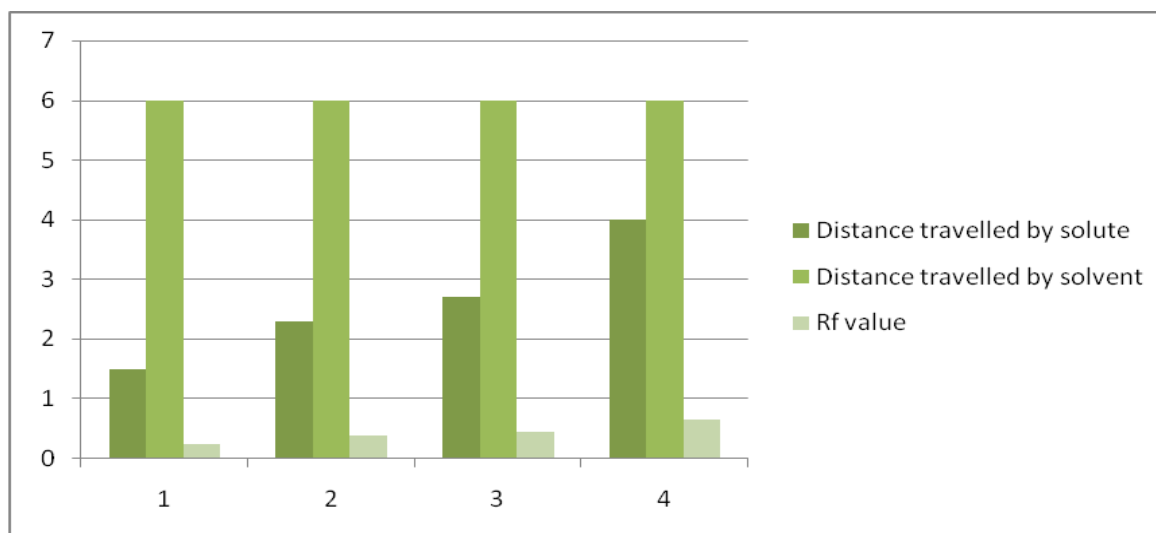


Fig. 4: TLC of flavonoids with solvent system II (nButanol: Ethanol: Water 4:1.5:5)

In TLC solvent system I the four spots were observed for important flavonoids after the visualization process, which was having RF values 0.26, 0.41, 0.46 and 0.61 respectively. Then with solvent system II, there were four spots also observed for flavonoids in *E. thymifolia* after the visualization process, which having RF values 0.25, 0.38, 0.45, and 0.66 respectively. This study reveals many antioxidant and anti-aging potential of *E. thymifolia* plant. The macroscopic and microscopic analysis conducted for *E. thymifolia* to get rid of mixing and adulteration of other medicinal plants having equal morphological characters. Phytochemicals showed good quality are having significant importance because these all phytochemicals are being used in indigenous system of medicine. The phytochemicals present in this medicinal plant may play an important role in human nutrition. These trace elements are required in the human body for building red blood cells and other body functioning [14]. Deficiency of these elements may cause hypertension, antibiotic sensitivity, hyperactivity, hyperglycemia, maniac disorders, insomnia, allergies and osteoporosis [15]. Thus this plant could serve as a good source of minerals when consumed. This confirmed the observation of some researchers who concluded that green vegetables are also a good source of phytochemicals [16]. We concluded that the aerial parts of plant contain good amount of phytochemicals. The distribution of these components in common medicinal plants has an important application for the health of people in addition to the basic need of developing countries. There is a great need to further research. Now

emerging applications of Ayurveda is coming up as Ayurveda biology, where all Ayurveda will be verified with all scientific parameters and techniques. So that through this national heritage of India, the whole world will gain advantage to be healthy on body, mind and spirit levels.

CONCLUSIONS

Euphorbia thymifolia is a plant, which contains so many phytochemical properties, medicinal uses for various therapeutic purposes. Glycosides are present in this plant which has played a major role in human health, entire plant parts are useful. In TLC analysis four spots were observed, which indicates that flavonoids are present in good quality this fact is also supported by quantitative analysis of flavonoids for *E. thymifolia*.

Future studies can be carried to find out the relations between climate changes on the amount of phytochemicals quantity and quality change of medicinal plants. This is ample need to work to improve the quality and quantity of these valued products for pharmaceutical formulation development. It is also observed that there is no patent so far on this plant. Therefore, further studies of standardization of extracts, isolation and identification of active constituents, mode of action, formulation development, clinical and toxicological efficacy remain to be explored.

ACKNOWLEDGMENTS

We take this opportunity to acknowledge sincere thanks to our Honorable chairman, Dr. S. C. Kulshreshtha, respected Executive Director Dr. B.K. Tyagi, Director Dr. R.

S. Saxena, Shri Ram Group of Colleges Muzaffarnagar, U.P. India for providing necessary facility and tools to carry out this research dissertation work for Post Graduate students of M. Sc. Biotechnology.

CONTRIBUTION OF AUTHORS

Substantial contributions to the conception or design of the work and drafting of the article were done by the Corresponding author, while the data collection, data analysis and interpretation for the work completed by remaining authors, critical revision of the article for important intellectual content were contributed by each author.

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Received: 13 Feb 2018/ Revised: 19 Mar 2018/ Accepted: 24 Apr 2018

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